Algal Research 9 (2015) 151–159

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

The effect of carbon dioxide rich environment on carbonic anhydrase activity, growth and metabolite production in indigenous freshwater microalgae

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ARTICLE INFO

Article history:
Received 15 October 2014
Received in revised form 22 January 2015
Accepted 15 February 2015
Available online xxxx

Keywords:
Microalgae
CO_2 fixation
Photobioreactor
Carbonic anhydrase
Dissolved inorganic carbon

ABSTRACT

Indigenous freshwater microalgae from natural habitats may have potential for bio-mitigation of atmospheric CO_2. The present study evaluates the response of three indigenous microalgal isolates viz., *Desmodesmus* sp., *Kirchneriella* sp. and *Acutodesmus* sp., to CO_2 rich environment (10, 20 and 30%) in a closed photobioreactor. In a time course study, levels of CO_2 in culture medium were observed to modulate the activity of carbonic anhydrase (CA), a key enzyme of carbon concentrating mechanism (CCM) of microalgae. The CA activity decreased on availability of free CO_2 and increased on depletion of free CO_2 in the culture medium. Maintenance of algal cultures in CO_2 rich environment for a period of 16 days enhanced the biomass concentration, specific growth rate, chlorophyll and carbon dioxide biofixation rate by 2–4 fold. Overall productivity, carbon, carotenoid and lipid contents also increased. Palmitic and oleic acids were the major fatty acids of the algal lipids. CO_2 rich environment affected the fatty acid profile in *Desmodesmus* sp. with an increase in unsaturated fatty acids.

1. Introduction

Increase in the levels of atmospheric CO_2, a leading cause of global warming, has been recognised as a great challenge to world sustainability [1]. Among the various CO_2 mitigation strategies, bio-fixation of CO_2 is considered to be a comparatively more sustainable strategy, both economically and environmentally [2]. Microalgae are recognised as a potential and efficient system for bio-mitigation of CO_2 from various sources like atmosphere, flue gases and chemically fixed soluble carbonates [3,4]. These photosynthetic microorganisms have high growth rates and high CO_2 fixation rates compared to slow growing conventional terrestrial and aquatic plants [4–8]. The biological mitigation of CO_2 is advantageous as the process of CO_2 fixation through photosynthesis leads to production of biomass energy [9–11]. Microalgal biomass contains significant amounts of lipids, carbohydrates, proteins and other valuable compounds, such as pigments, minerals and vitamins. Therefore, microalgal biomass could be a source of active ingredients for food, feed, pharmacy, cosmetic, biodiesel, etc. [3,12–15]. The utilisation of CO_2 into value added products would reduce the sequestration cost [16]. Therefore, a successful strategy of CO_2 bio-mitigation by microalgae would depend on identifying the microalgae which tolerate higher levels of CO_2, exhibit higher biomass productivity with potential for value added products and/or high lipid content for production of biodiesel.

The lipid profile of a majority of oleaginous algal species is similar to vegetable oils derived from terrestrial plants suitable for biodiesel [17]. Fatty acids are synthesized by algae primarily for esterification into glycerol based membrane lipids which constitute about 5–20% dry cell weight. Membrane glycerolipids of algae include medium chain (C10–C14), long chain (C16–C18) and very long chain (>C20) fatty acid species [18,19]. Microalgae can modify their lipid biosynthesis pathway, under unfavourable environment or stress conditions, towards the formation and accumulation of neutral lipids [20]. The neutral lipids may form 20–50% of dry cell weight, mainly in the form of triacylglycerol (TAG) [18,19]. The ability of microalgae to synthesize and accumulate high amounts of neutral lipids makes them suitable as feedstock for biodiesel production [19,21]. Fatty acid composition of typical microalgal oil shows a mixture of unsaturated fatty acids such as palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2) and linolenic acid (C18:3) with some quantities of saturated fatty acid such as palimitic (C16:0) and stearic acid (C18:0) [22]. The oleic acid is known to be a main component of biodiesel [23] and the addition of methyl oleate has been suggested to improve the properties of biodiesel fuel, such as its oxidative stability and low melting temperature [24]. Therefore, microalgal lipids, which are rich in oleic acid, could be favoured as feedstock for biodiesel production.

The level of CO_2 has a significant effect on microalgae as it affects the medium pH and also the availability of bicarbonate used by microalgae as carbon source [25]. The response of microalgae to higher levels of CO_2 appears to vary from species to species. Some of the microalgae like *Chlorella kessleri* and *Scenedesmus obliquus* were best grown under 6,

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http://dx.doi.org/10.1016/j.algal.2015.02.014
2211-9264/

12 and 18% CO_2 [10] and some of them like *Chlorella* sp. [26] and *Nannochloropsis oculata* [27] were completely inhibited at above 5% of CO_2. *Chlorella pyrenoidosa* and *S. obliquus* have also been reported to grow well under CO_2 concentration of 5–20% [28].

Very few studies have reported the effect of varying levels of CO_2 on lipid content and fatty acid profile of microalgae. In a study of biomass productivity and lipid content of three microalgae under high levels of CO_2, Yoo et al. [23] suggested that *Scenedesmus* sp. could be selected due to its high biomass productivity and *Botryococcus braunii* for its high lipid content suitable for biodiesel production. Carbon dioxide had no effect on lipid content of marine diatom *Chaetoceros* cf. *wighamii* [29]. Chiu et al. [27] reported that both biomass and lipid productivity and lipid content of *N. oculata* cultures decreased when aerated with the CO_2 concentrations of >2%. The decrease may be attributed to the reduction in medium pH at higher levels of CO_2. It has been reported that the carbon assimilation of lipid synthesis decreases with decreasing pH [30] as the availability of bicarbonate for carboxylation of lipid synthesis reduces at lower pH. Gordillo et al. [31] studied the effect of 1% CO_2 on *Dunaliella viridis* and found no significant difference in total lipid and total neutral lipid content compared to cultures supplied with atmospheric levels of CO_2. However, the triglyceride levels almost doubled in cultures supplied with 1% CO_2 and an increase in total phospholipids was also seen. Both, the high and low levels of CO_2, have been shown to affect the unsaturation in fatty acids. Higher levels of CO_2 enhanced the unsaturation in fatty acids of freshwater microalga, *Scenedesmus* sp. [32]. While on the contrary, an increase in polyunsaturated fatty acids in coccolithotroph *Emiliania huxleyi* was associated with the lower levels of CO_2 [33].

Increased levels of atmospheric CO_2 are expected to increase the algal biomass in natural aquatic environment through enhanced removal of atmospheric CO_2 by algae [34]. In microalgae a light energy dependent uptake and build up of HCO_3^- is facilitated by carbon concentrating mechanism (CCM) inside the pyrenoids which are densely packed with Rubisco. In pyrenoids, the carbonic anhydrase catalyses the formation of CO_2 and facilitates its concentration in the close vicinity of Rubisco, compensating the low affinity of Rubisco for CO_2 [34,35]. The CO_2 levels, therefore, may have a modulating effect on CCM of microalgal cultures, and the effect may be exhibited by the activity of carbonic anhydrase, the key enzyme of CCM of microalgae.

Most of the studies on the effect of elevated levels of external CO_2 on phytoplankton have been carried out in the oceanic environment [36–39] and there is a need to study the effect of elevated levels of atmospheric CO_2 on freshwater microalgae [40]. It is important to study the growth rate, productivity and CO_2 bio-fixation rate of microalgae under various levels of CO_2 to evaluate their potential for CO_2 sequestration [4,10] through large scale cultivation. Indigenous microalgae isolated from natural habitats with the ability to adapt to climatic and environmental changes of the region may be more suitable for field applications; however, studies on the influence of CO_2 in indigenous microalgae have been limited.

The present study evaluates the effect of different levels of CO_2 on modulation of carbonic anhydrase enzyme activity, growth, CO_2 bio fixation rate, chlorophyll, carotenoid, lipid content and fatty acid profile in three indigenous freshwater microalgal isolates, viz., *Desmodesmus* sp., *Kirchneriella* sp. and *Acutodesmus* sp.

2. Material and methods

2.1. Microalgal cultures: isolation, identification and culture conditions

The three indigenous freshwater microalgal isolates, viz., *Desmodesmus* sp., *Kirchneriella* sp. and *Acutodesmus* sp. used in the present study were isolated from different natural freshwater bodies of southern part of India. The details of geographical location and coordinates of the water bodies, from which the microalgae were isolated, are provided in Table 1. The method described by Vidyashankar et al. [32] was followed for isolation and purification of microalgal isolates. The Knop's solution was used for enrichment of microalgal samples and further purification was achieved by serial dilution and plating on solidified Bold's basal medium. Repeated plating in antibiotic containing medium was carried out to obtain axenic cultures. The purified individual colonies of microalgal isolates named as isolates I-1, I-2 and I-3 were isolated and maintained in sterile BG11 medium [41] at 25 ± 1 °C under 30 μE m^{-2} s^{-1} light intensity with 16:8 h light and dark cycle. The same medium and the growth conditions were used for the experimental studies.

Microalgal isolates were identified based on the morphological characteristics as described by Philipose [42] and through molecular technique. Morphological characteristics were enumerated by a light microscope (Olympus BX 51, Japan) and cell dimensions were measured with a camera and imaging software (ProgRes C5, Germany) (Table 1, Fig. 1). Genomic DNA was isolated from the algae and the ITS 2 region was amplified using the primers, forward- 5′CTGCGGAAGGATCATTGAAT3′ and reverse- 5′AGCGGGTAGTCTTGCCTGAG 3′. PCR was carried out in thermal cycler (Bio-Rad, USA) with initial denaturation at 95 °C for 5 min, 30 cycles of 95 °C for 1 min, 55 °C for 45 s and 72 °C for 45 s and a final extension step at 72 °C for 10 min. PCR products were purified using MinElute PCR Purification kit (Qiagen, Germany), according to the manufacturer's protocol and sequenced (Bioserve, Hyderabad, India). The sequences were analysed using similarity blast search with the help of NCBI database (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The microalgal isolates are being perpetually maintained at CSIR-CFTRI by regular subculturing.

The isolates were screened for their tolerance to elevated levels of CO_2 by studying their growth profile under varying levels of CO_2 (up to 30% v/v) in closed photobioreactors of low density poly ethylene (LDPE) sleeves.

2.2. Estimation of dissolved inorganic carbon (DIC) species in algal culture medium

Dissolved inorganic carbon species in terms of free CO_2, carbonate and bicarbonate alkalinity, were measured in culture medium using titrimetric methods as described in APHA and Snoeyink and Jenkins [43,44]. Culture media samples were obtained by removing the algal cells by centrifugation. For estimation of free CO_2, the 50 mL of sample was titrated with titrant (0.02 N NaOH) to the end-point pH of 8.3 using Phenolphthalein indicator. For estimation of alkalinity, the 50 mL sample was titrated with 0.02 N H_2SO_4. First the Phenolphthalein alkalinity was estimated by titrating the sample with standard acid to the end point pH of 8.3 using Phenolphthalein indicator. The titration was continued to the end point pH of 4.5 using Bromocresol green indicator to

Table 1
Morphological characteristics and habitat description of the microalgal isolates.

Sl. no.	Microalgae	Morphological description	Cell dimensions	Geographical location of natural habitat
1.	*Desmodesmus* sp. (I-1)	Cells predominantly tetrad, thick pyrenoid present, mucilaginous sheath absent	6.39 ± 0.05 (Length) × 5.64 ± 0.25 (Width) μm	Karanji lake, Mysore 12°18′17.02″N, 76°40′34.40″E, elevation 749 m
2.	*Kirchneriella cornuta* (I-2)	Cells unicellular, spindle/lunate shaped	7.75 ± 1.13 (L) × 1.91 ± 0.51 (W) μm	Raghavendra swami temple pond, Mantralayam, A.P. 15°56′32.88″N, 77°25′25.13″E, elevation 323 m
3.	*Acutodesmus* sp. (I-3)	Unicellular, Round	4.12 ± 0.34 μm	Karanji lake, Mysore